Bulgarian Chemical Communications, Volume 46, Number 4 (pp. 831 – 839) 2014

GC-MS profiling, antioxidant, and antimicrobial studies of various parts of *Carissa grandiflora*

M. Abbas[1], N. Rasool[1,*], M. Riaz[2], M. Zubair[1], M. Abbas[3], Noor-Ul-Haq[1], N. Hayat[1]

[1] *Department of Chemistry, Government College University Faisalabad 38000, Pakistan*
[2]*Department of Chemistry, University of Sargodha Women Campus, Faisalabad, Pakistan*
[3] *Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan*

Received, November 5, 2013; Revised January 2, 2014

The present research work was carried out to evaluate the bioactivity of methanol extracts of leaves, roots and stems of *Carissa grandiflora* and their fractions in solvents of different polarity (*n*-hexane, chloroform, ethyl acetate and *n*-butanol). The extracts and their fractions contained appreciable levels of total phenolic contents (TPC) ranging from 31.17 to 349.43 Gallic Acid Equivalent (GAE mg/100 g) of dry matter (leaves), 38.85 to 269.81 GAE, mg/100 g of dry matter (roots) and 40.18 to 241.11 GAE, mg/100 g of dry matter (stems). Total flavonoid contents were found to be from 59.14 to 284.99, 32.27 to 199.74 and 21.37 to 158.56 Catechin Equivalent (CE mg/100 g) of dry matter for leaves, roots and stems, respectively. IC_{50} values in case of DPPH radical scavenging activity of leaves, roots and stems were from 20.89 to 578.9, 12.28 to 325.31 and 6.15 to 941.4 μg/mL respectively. The percentage inhibition of peroxidation in a linoleic acid system was from 11.34 to 46.7, 15.56 to 41.31 and 18.14 to 50.46 for leaves, roots and stems, respectively. The methanol extracts of all three parts exhibited the maximum reducing activity in comparison to other fractions. Maximum antibacterial activity was shown by the ethyl acetate fraction of stems against *S. aureus*, its *n*-butanol fraction against *E. coli* and its methanol extract against *S. epidermidis*. *C. albicans* revealed the highest resistance against the ethyl acetate fraction of roots. GCMS analysis of the *n*-hexane fraction of roots revealed that this part of the plant is enriched with the maximum number of bioactive compounds.

Keywords: *Carissa grandiflora*, linoleic acid, phenolics, flavonoids, DPPH, gallic acid

INTRODUCTION

Plants containing a wide variety of ingredients are being used in traditional medicines. These medicines are used to treat infectious, as well as chronic diseases. The medicinal plants contain some secondary metabolites which produce a characteristic physiological action on the human body [1]. The most significant compounds present in plants are phenolic compounds like tannins, flavonoids and alkaloids [2].

Carissa grandiflora is a shrub of high ornamental value. Its large, lush green, thick, shiny leaves are very showy and attractive and the white star-shaped flowers are fragrant. It can be grown in containers and makes an ideal container specimen. It can also be used as a large dense security hedge or barrier due to its large thorns which are practically impenetrable. Its oval to elliptical shaped radish fruits are edible and are very delicious in taste. The cranberry-flavoured fruits are used in sauces, cakes, desserts, jams, jellies, yogurt and ice cream. The plant is also used to make graceful and elegant bonsai specimen [3]. Plants belonging to this family are of immense medicinal importance. Several authors have analyzed the chemical and biological properties of some of these plants [4-6] and more research work should be dedicated to the unexplored plants.

In the present research work we have made an attempt to analyze the biological (antioxidant and antimicrobial) activity of methanol extracts of leaves, roots and stems of *Carissa grandiflora*.

EXPERIMENTAL

Collection of plant

The selected plant *Carissa grandiflora* was collected from the Madina Nursery Tehsil Pattoki District Kasur and was identified by Dr. R.B. Tareen from the Department of Botany, University of Balochistan, Quetta, Pakistan. A voucher specimen (CG-NR-05) was deposited in the herbarium/collection of the Department of Botany, University of Balochistan, Quetta, Pakistan.

Preparation of methanol extracts

Plant extracts from leaves, roots and stems were prepared by the soaking method. According to this method, portions of finely ground leaves (488 g), roots (156 g) and stems (466 g) were placed in separate flasks and measured volumes of methanol

* To whom all correspondence should be sent:
E-mail: nasirhej@yahoo.co.uk

were added to each flask. Then the flasks were kept at room temperature for 4 to 5 days and were shaken at regular intervals. Vacuum rotary evaporator (Eyela, Tokyo Rikakikai Co., Ltd Japan) was used to evaporate the solvent under vacuum at 45ºC. In this way, viscous extracts were obtained which were dried and stored at -4ºC. Sufficient amounts of methanol extracts (18.02 g leaves, 8.06 g roots and 16.67 g stems) were obtained by repeating the extraction process thrice. The methanol extracts of leaves, roots and stems were dissolved in distilled water separately and then fractionation was carried out using solvents of different polarity. The solvents used for fractionation of the methanol extracts were *n*-hexane, chloroform, ethyl acetate and *n*-butanol [7].

Phytochemical analysis

Phytochemical screening of the methanol extracts of leaves, roots and stems was performed according to a previously described method [8,9]

Total Phenolic Contents (TPC)

TPC of leaves, roots and stems extracts of the plant and their fractions were determined using the Follin-Ciocalteu reagent method [10,11].

Total Flavonoid Contents (TFC)

TFC of extracts/fractions of leaves, roots and stems were determined spectrophotometrically following a previously reported method [12].

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out spectrophotometrically as described in [13]. Stock solution was prepared by dissolving 100 mg of each extract or fraction in 100 mL of methanol. From the stock solution concentrations in the range 0.2-1 mg/mL were made. To each concentration, 5 mL of freshly prepared DPPH of concentration 0.025 g/L (0.0050 g DPPH in 200 mL CH_3OH) was added. After 10 min, the absorbance of the resulting solution and the blank (5 mL DPPH + 1 mL methanol) was measured at 515 nm. Three replicates were recorded for each sample. The inhibitory effect of DPPH was calculated according to the following equation:

$$Inhibition\ (\%) = 100 \times (A_{blank} - A_{sample} / A_{blank})$$

where A_{blank} is the absorbance of the control (containing all reagents except the test samples), and A_{sample} is the absorbance of the test samples. IC_{50} value (mg/mL), defined as the concentration at which the scavenging activity was 50% and caused 50% neutralization of DPPH radicals, was measured from the plot of concentration *versus* percentage inhibition.

Determination of reducing power

The reducing power of methanol extracts and fractions of leaves, roots and stems was evaluated spectrophotometrically [11,14]. Stock solution was prepared by dissolving 100 mg of each extract or fraction in 100 mL of methanol. From the stock solution different concentrations in the range of 0.2-1 mg/mL were made. To 1 mL of each concentration, 2 mL phosphate buffer and 2 mL potassium ferricyanide (1%) were added. The mixture was incubated at 50ºC for 20 min. Then 2 mL of 10% trichloroacetic acid were added and the mixture was centrifuged at 3000 rpm for 10 min at 5ºC. The upper layer of the solution was removed. Finally, 5 mL deionised water and 1 mL $FeCl_3$ were added. The absorbance of the reaction mixture was measured at 700 nm using a spectrophotometer. Three replicates were measured for each sample.

Antioxidant activity determination in a linoleic acid system

The antioxidant potential of the extracts/fractions of leaves, roots and stems was estimated following an already reported method [15,14].

Antimicrobial assay of plant extracts (leaves, roots and stems) and their fractions

Disc Diffusion Method

Antimicrobial activity of the methanol extracts and different fractions was examined by the disc diffusion method [16,17]. The discs (5 mm diameter) were impregnated with 10 mg/mL extracts/fractions (50 µL/disc) and were placed on inoculated agar under aseptic conditions. Discs injected with 100 µL of the respective solvent served as negative controls; Amikacin (50 µL/disc) and Terbinaline (50 µL/disc) were employed as positive references for bacteria and fungi, respectively. The petri dishes were incubated at 37 ± 0.1°C for 20-24 hours and at 28±0.3°C for 40-48 hours for bacteria and fungi, respectively. The inhibition zones formed around each disc were measured at the end of the specified period with a zone reader. Zone inhibition diameter (ZID) values were directly related with the antimicrobial activity of the extracts/fractions. Determination of the inhibitory properties was carried out in triplicate.

Resazurin Microtitre-Plate Assay of minimum inhibitory concentration MIC

The minimum inhibitory concentration (MIC) of

the plant extracts/fractions was determined by the resazurin microtitre-plate assay reported in [7,18].

Sample Preparation for GC-MS analysis

An amount of 100 g of the dried and ground plant was extracted with *n*-hexane in a Soxhlet apparatus for the GC-MS analysis [1].

Gas Chromatography/Mass Spectrometry Analysis

The GC-MS analysis of the *n*-hexane fractions of leaves, roots and stems were carried out using a GC 6850 Network gas chromatographic system equipped with 7683 B series auto injector and 5973 inert mass selective detector (Agilent Technologies USA). The compounds were separated on an HP-5 MS capillary column using 5% phenyl polysiloxane as stationary phase, column length 30.0 m, internal diameter 0.25 mm and film thickness 0.25 μm. The injector temperature was 300°C. 1.0 μL of the sample was injected in split mode with a split ratio of 30:1. Helium with a flow rate of 1.5 mL/min was used as a carrier gas. The temperature program was: initial temperature 150°C, hold for 1 min at this temperature; ramp at a rate of 10°C/min up to 290°C, hold for 5 min at this temperature. The temperature of the MSD transfer line was 300°C. Mass spectra were recorded in electron ionization (EI) mode with ionization energy of 70 eV; the mass range scanned was 3–500 *m*/*z*. The temperature of the ion source was 230°C and that of the MS quadrupole 150°C. The identification of the components was based on comparison of their mass spectra with those of the NIST mass spectral library with some modification [19,20].

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytochemical constituents of the methanol extracts were analyzed and the results are given in Table 1. Alkaloids and terpenoids were found to be present in all parts of the plant. There were no flavonoids in the leaves while steroids and tannins were found in leaves and roots, respectively.

Table 1: Phytochemical analysis of methanol extracts

Plant part	Alkaloids	Steroids	Flavonoids	Tannins	Terpenoids
Leaves	+	+	-	-	+
Roots	+	-	+	+	+
Stems	+	-	+	-	+

Percent yields of methanol extracts and different fractions of leaves, roots and stems of *Carissa grandiflora*

The percent yields of the plant methanol extracts and organic fractions are shown in Table 2.

Table 2: Percent yield of methanol extracts and various organic fractions of leaves, roots and stems

Extracts/Fractions	Yield (g/100g)		
	Leaves	Roots	Stems
Methanol	13.69	10.06	12.58
n-Butanol	3.87	3.62	4.54
Ethyl acetate	3.54	4.34	2.47
Chloroform	4.63	2.49	2.36
n-Hexane	1.82	1.2	2.48

The highest amounts were extracted with methanol from all parts of the plant followed by the chloroform fraction of the leaves (4.6 g) and *n*-butanol fraction of the stems (5.45 g). *n*-Hexane was found to be the least effective solvent. The amount of substances that can be extracted from a plant depends upon the nature and amount of solvent and the mixing procedure used. Sample to sample variation in extracted material is possible [11].

We determined the total phenolic contents (TPC) and the total flavonoid contents (TFC) in the methanol extracts and different fractions of *C. grandiflora* roots, leaves and stems. Total phenolic contents were expressed as gallic acid equivalents (GAE), mg/100 g of dry matter. The amounts of TPC extracted from leaves, roots and stems were in the ranges of 31.17 to 349.4, 38.85 to 269.81 and 40.18 to 241.11 GAE (mg/100 g of dry matter), respectively (Table 3). Total flavonoid contents were expressed as mg catechin equivalents (CE) per 100 g of dry matter. The quantities of TFC obtained from leaves, roots and stems were in the range of 59.14 to 284.99, 32.27 to 199.74 and 21.37 to 158.56 CE (mg/100 g of dry matter), respectively.

Effect of polarity on the extraction of TPC and TFC has been illustrated in numerous reports. Soil and growing conditions have drastic effects on the amount of TPC which can be extracted from the plant. The capability of a given solvent to dissolve endogenous substances determines the amounts of TPC and TFC extracted from the plant. The highest quantity of phenolic compounds was extracted by methanol while n-hexane, owing to its non-polar nature, was found to be the least effective solvent for extraction of phenolics.

DPPH scavenging assay

We investigated the free radical scavenging activity of methanol extracts and fractions of *Carissa grandiflora* leaves, roots and stems. Free radical scavenging activities were measured by DPPH assay. The methanol extracts and various fractions of the plant showed excellent radical quenching activities having IC_{50} values of 20.89 to 578.9, 12.28 to 325.31 and 6.25 to 941.4 mg/mL for

Table 3: Phytochemical and antioxidant studies of methanol extracts and different fractions of leaves, roots and stems of *Carissa grandiflora*

Assay	Extract and fractions	Part		
		Stems	Roots	Leaves
TPC (GAE, mg/100g)	Methanol	241.11 ± 1.35^{c}	269.81 ± 1.64^{b}	349.43 ± 2.23^{a}
	n-Butanol	163.63 ± 1.36^{f}	184.24 ± 1.18^{e}	211.29 ± 0.48^{d}
	Ethyl acetate	105.58 ± 0.40^{i}	135.69 ± 0.71^{g}	117.23 ± 0.58^{h}
	Chloroform	59.36 ± 0.39^{k}	103.05 ± 0.53^{i}	69.87 ± 0.49^{j}
	n-Hexane	40.18 ± 0.27^{l}	38.85 ± 0.39^{l}	31.17 ± 0.20^{m}
TFC (CE, mg/100g)	Methanol	158.56 ± 1.14^{c}	199.74 ± 1.20^{b}	284.99 ± 1.69^{a}
	n-Butanol	83.43 ± 0.68^{h}	132.21 ± 0.76^{d}	156.9 ± 0.87^{c}
	Ethylacetate	75.78 ± 0.29^{i}	91.54 ± 0.52^{g}	114.44 ± 0.76^{e}
	Chloroform	62.27 ± 0.29^{j}	52 ± 0.59^{k}	101.79 ± 0.69^{f}
	n-Hexane	21.37 ± 0.15^{m}	32.27 ± 0.25^{l}	59.14 ± 0.33^{j}
IC_{50}	Methanol	6.15 ± 0.02^{m}	12.28 ± 0.35^{l}	20.89 ± 0.40^{i}
	n-Butanol	18.27 ± 0.01^{j}	14.68 ± 0.04^{k}	72.94 ± 0.16^{e}
	Ethyl acetate	26.09 ± 0.13^{h}	29.74 ± 0.34^{g}	76.8 ± 0.11^{d}
	Chloroform	28.49 ± 0.25^{g}	41.45 ± 0.14^{f}	78.43 ± 0.32^{d}
	n-Hexane	941.4 ± 0.80^{a}	325.31 ± 0.64^{c}	578.9 ± 0.90^{b}
% Inhibition of linoleic acid peroxidation	Methanol	50.46 ± 0.27^{a}	41.31 ± 0.49^{c}	46.47 ± 0.27^{b}
	n-Butanol	39.96 ± 0.38^{c}	33.2 ± 0.18^{e}	37.89 ± 0.33^{d}
	Ethyl acetate	33.1 ± 0.52e^{f}	31.34 ± 0.68^{f}	31.84 ± 0.25e^{f}
	Chloroform	28.57 ± 0.26^{g}	23.82 ± 0.12i	26.21 ± 0.17^{h}
	n-Hexane	18.14 ± 0.24^{j}	15.56 ± 0.48^{k}	11.34 ± 0.09^{l}

The values are the average of triplicate samples (n=3) ± S.D., (p <0.05)
The superscript alphabets showed significant differences.

leaves, roots and stems, respectively. Methanol extracts of leaves, roots and stems exhibited the lowest IC_{50} values (20.89, 12.28 and 6.25 µg/mL) followed by *n*-butanol fractions (72.94, 14.68 and 18.27 µg/mL), ethyl acetate (76.8, 29.7 and 20.09 µg/mL), chloroform (78.43, 41.45 and 28.49 µg/mL) and *n*-hexane (578.9, 325.31 and 941.4 µg/mL), respectively. IC_{50} values indicated that the methanol extracts display the highest free radical scavenging activity while n-hexane fractions display the lowest one.

Percent inhibition of linoleic acid peroxidation

The percent inhibition of linoleic acid peroxidation by crude extracts/fractions of leaves, roots and stems is shown in Table 3. The values are in the range of 18.14% to 50.46% for stems, 15.56% to 41.31% for roots and 11.34% to 46.47% for leaves. The methanol extract and the n-butanol fraction of leaves exhibited excellent inhibition of linoleic acid oxidation, i.e. 46.47 and 37.89, respectively. Other fractions also showed reasonable inhibition. The methanol extracts of all three parts exhibited the highest inhibition of linoleic acid oxidation followed by *n*-butanol, ethyl acetate, chloroform and *n*-hexane fractions. On comparing the three plant parts, the methanol extract and fractions obtained from stems showed better inhibition than those of the other two parts.

Reducing power

Antioxidant activity can be determined by evaluating the reducing power of methanol extracts of leaves, roots and stems of plant and different fractions. The reducing potential of leaves, roots and stems was measured at a concentration of 0.2-1.0 mg/mL. The results showed that the absorbance increases with concentration. The assay of the reducing power of all fractions of the three parts showed a linear increase of absorbance with concentration. Maximum absorbance values (1.73, 1.82 and 1.82) were shown by the methanol extracts of leaves, roots and stems compared with other fractions.

The values for the methanol extracts of leaves, roots and stems range from 0.83 to 1.73, 0.94 to 1.82 and 0.97 to 1.82, respectively. *n*-Hexane fractions of all three parts exhibited the lowest reducing activity.

Antimicrobial Activity

Antimicrobial activity of methanol extracts and different fractions of leaves, roots and stems against a panel of pathogenic microorganisms was assessed by the disc diffusion method. The results are given in Table 4. The extracts and fractions of stems, roots and leaves were tested against three bacterial and one fungal strain. The different fractions and methanol extracts of all parts of the plant revealed a broad spectrum of activities by forming clear zones

Table 4: Antimicrobial activity of methanol extracts and different fractions of leaves, roots and stems of *Carissa grandiflora*

Strain	Extract and fractions	Plant part		
		Stems	Roots	Leaves
S. aureus	Methanol	7.81 ± 0.01^{g}	7.73 ± 0.02^{gh}	6.82 ± 0.02^{l}
	n-Butanol	7.52 ± 0.02^{i}	8.65 ± 0.02^{c}	8.27 ± 0.01^{e}
	Ethyl acetate	8.73 ± 0.02^{c}	7.69 ± 0.02^{h}	7.15 ± 0.02^{j}
	Chloroform	8.53 ± 0.01^{d}	6.56 ± 0.04^{m}	6.23 ± 0.01^{n}
	n-Hexane	7.17 ± 0.02^{j}	6.63 ± 0.02^{m}	6.95 ± 0.02^{k}
	Amikacin	13.55 ± 0.05^{a}	9.41 ± 0.01^{b}	8.41± 0.01^{f}
E. coli	Methanol	7.49 ± 0.04^{jk}	7.33 ± 0.01^{kl}	8.42 ± 0.02^{e}
	n-Butanol	9.03 ± 0.03^{d}	7.28 ± 0.01^{l}	7.65 ± 0.01^{i}
	Ethyl acetate	7.93 ± 0.01^{h}	7.57 ± 0.01^{ij}	8.10 ± 0.04^{fg}
	Chloroform	8.41 ± 0.01^{e}	8.25 ± 0.06^{f}	8.05 ± 0.03^{gh}
	n-Hexane	7.45 ± 0.04^{jk}	7.20 ± 0.04^{l}	7.51 ± 0.01^{ij}
	Amikacin	10.72 ± 0.02^{c}	11.03 ± 0.04^{b}	12.34 ± 0.04^{a}
S. epidermidis	Methanol	10.82 ± 0.04^{d}	10.27 ± 0.01^{e}	9.68 ± 0.05^{h}
	n-Butanol	9.52 ± 0.01^{i}	9.93 ± 0.03^{g}	9.22 ± 0.04^{j}
	Ethyl acetate	9.56 ± 0.02^{hi}	8.82 ± 0.02^{k}	7.64 ± 0.02^{n}
	Chloroform	8.65 ± 0.02^{l}	10.09 ± 0.04^{f}	8.19 ± 0.03^{m}
	n-Hexane	9.3 0± 0.01^{j}	9.88 ± 0.02^{g}	8.53 ± 0.02^{l}
	Amikacin	11.15 ± 0.02^{c}	11.69 ± 0.01^{b}	12.47 ± 0.05^{a}
C. albicans	Methanol	10.67 ± 0.03 ^{i}	13.72 ± 0.02 ^{d}	10.9 ± 0.04^{hi}
	n-Butanol	10.78 ± 0.01 ^{hi}	12.57 ± 0.05 ^{f}	13.2 ± 0.12^{e}
	Ethyl acetate	15.17 ± 0.06 ^{a}	8.16 ± 0.04 ^{m}	11.12 ± 0.05^{gh}
	Chloroform	10.17 ± 0.02 ^{j}	10.12 ± 0.02 ^{j}	9.53 ± 0.02^{k}
	n-Hexane	8.64 ± 0.03 ^{l}	10.16 ± 0.02 ^{j}	11.53 ± 0.23^{g}
	Terbinaline	15.73 ± 0.06 ^{b}	14.44 ± 0.06 ^{c}	14.51 ± 0.14^{c}

The values are the average of triplicate samples (n=3) ± S.D., ($p < 0.05$)
The superscript alphabets showed significant differences.
The methanol extract and fractions were analyzed at 5 mg/ml and Terbinaline at 1 mg/ml

of inhibition against strains. Results indicated that the *n*-butanol fraction of roots and the ethyl acetate fraction of stems showed good activity against *S. aureus* with inhibition zones of 8.65 and 8.73 mm, respectively. Maximum activity against *E. coli* was displayed by the methanol extract of leaves and the *n*-butanol fraction of stems with inhibition zones of 8.42 and 9.03 mm, respectively. Methanol extracts of leaves, roots and stems were found to be more effective against *S. epidermidis*, as compared to other fractions with inhibition zones (10.82, 10.27, 9.68 mm, respectively). Highest potential against *C. albicans* was shown by the ethyl acetate fraction of stems with inhibition zone of 15.73 mm. The antimicrobial activity for stems was found to be in the range of 7.17 (*n*-hexane) to 15.73 mm (ethyl acetate). For roots, the range was from 6.56 (*n*-hexane) to 13.72 mm (*n*-butanol) and for leaves from 6.23 (chloroform) to 13.2 mm (*n*-butanol). All extracts and fractions showed considerable activity against these strains. Methanol extract of roots and ethyl acetate fraction of stems showed strong activity against *C. albicans* with ZID values of 13.73 and 15.83 mm, respectively. The *n*-hexane, chloroform and *n*-butanol fractions of leaves exhibited moderate values of ZID with a maximum value for *n*-butanol (13.2 mm against *C. albicans*). The n-hexane fraction of roots showed poor activity with a maximum ZID value of 10.16 mm against *C. albicans*. Chloroform fraction of leaves showed minimum activity (6.23 mm) against *S. aureus*. All extracts and fractions were found particularly effective against *C. albicans* with inhibition zones ranging from 8.16 to 15.73 mm. The ethyl acetate fraction of stems exhibited specifically strong activity against *C. albicans* with ZID of 15.73 mm.

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the minimum concentration that could inhibit the growth of pathogens. The MIC activity of methanol extracts and different fractions of leaves, roots and stems of *C. grandiflora* against one fungal and three bacterial strains was evaluated using a modified resazurin microtitre-plate assay. MIC values were found to be inverse to the antimicrobial activity values. The MIC values are presented in mg/mL (Table 5).

Methanol extracts and fractions of stems, roots and leaves displayed MIC values in the range of

Table 5: Minimum inhibitory concentration (MIC), mg/ml, of methanol extracts and different fractions of leaves, roots and stems of *Carissa grandiflora*

Strains	Extract and fractions	Plant part								
		Stems			Roots			Leaves		
S. aureus	Methanol	1.41	±	0.03 ^{g}	0.89	±	0.01 ^{i}	1.35	±	0.02 ^{gh}
	n-Butanol	1.55	±	0.03 ^{d}	0.82	±	0.01 ^{de}	0.67	±	0.02 ^{de}
	Ethyl acetate	1.31	±	0.03 ^{ab}	1.23	±	0.01 ^{b}	0.88	±	0.01 ^{i}
	Chloroform	1.35	±	0.02 ^{a}	1.7	0±	0.01 ^{c}	1.46	±	0.05 ^{de}
	n-Hexane	1.72	±	0.03 ^{f}	1.43	±	0.01 ^{ef}	1.24	±	0.02 ^{de}
	Amikacin	0.95	±	0.02 ^{hi}	0.51	±	0.01 ^{j}	0.43	±	0.02 ^{j}
E. coli	Methanol	1.83	±	0.02 ^{c}	0.74	±	0.02 ^{klm}	0.39	±	0.01 ^{jkl}
	n-Butanol	0.73	±	0.01 ^{fg}	0.85	±	0.02 ^{ef}	0.89	±	0.01 ^{e}
	Ethyl acetate	1.52	±	0.02 ^{hij}	0.63	±	0.01 ^{d}	0.65	±	0.01 ^{gh}
	Chloroform	1.24	±	0.07 ^{b}	0.56	±	0.02 ^{e}	0.76	±	0.02 ^{ijk}
	n-Hexane	5.52	±	0.07 ^{a}	0.91	±	0.01 ^{e}	0.93	±	0.01 ^{hi}
	Amikacin	0.27	±	0.01 ^{lm}	0.23	±	0.01 ^{m}	0.31	±	0.01 ^{klm}
S. epidermidis	Methanol	1.88	±	0.06 ^{d}	0.24	±	0.01 ^{j}	0.56	±	0.02 ^{i}
	n-Butanol	2.41	±	0.01 ^{de}	0.92	±	0.02 ^{gh}	0.78	±	0.01 ^{h}
	Ethyl acetate	2.19	±	0.01 ^{c}	0.97	±	0.01 ^{i}	0.95	±	0.02 ^{f}
	Chloroform	2.69	±	0.01 ^{b}	0.90	±	0.01 ^{fg}	0.87	±	0.02 ^{i}
	n-Hexane	2.61	±	0.02 ^{a}	0.94	±	0.01 ^{f}	0.82	±	0.02 ^{gh}
	Amikacin	1.75	±	0.01 ^{e}	0.23	±	0.01 ^{j}	0.27	±	0.01 ^{j}
C. albicans	Methanol	0.84	±	0.01 ^{g}	0.23	±	0.02 ^{i}	1.02	±	0.01 ^{g}
	n-Butanol	0.74	±	0.02 ^{f}	0.81	±	0.01 ^{de}	0.76	±	0.01 ^{ef}
	Ethyl acetate	0.55	±	0.02 ^{cd}	0.97	±	0.01 ^{g}	0.98	±	0.01 ^{a}
	Chloroform	0.86	±	0.02 ^{d}	0.91	±	0.01 ^{bc}	1.24	±	0.01 ^{g}
	n-Hexane	0.95	±	0.01 ^{ef}	0.87	±	0.01 ^{ab}	0.81	±	0.01 ^{de}
	Terbinaline	0.39	±	0.01 ^{h}	0.21	±	0.01^{i}	0.25	±	0.01 ^{i}

The values are the average of triplicate samples (n=3) ± S.D., (p <0.05)
The superscript alphabets showed significant differences.

0.52 to 5.52, 0.23 to 1.83 and 0.39 to 1.46 mg/mL respectively. *n*-Hexane fraction of stems exhibited the maximum MIC value (5.52 mg/mL). Methanol extracts of stems, roots and leaves showed maximum antimicrobial activity and the range of their MIC values was found to be from 0.23 to 1.88 mg/mL which means that it might show antimicrobial activity at this low concentration. MIC ranges of the *n*-hexane fraction were 0.91–1.43 mg/mL for roots, 0.53 –1.44 mg/mL for leaves and 0.75-5.52 mg/mL for stems. The MIC values revealed that the greater the antimicrobial activity, the lower would be the MIC value.

The minimum inhibitory concentration of the methanol extracts of roots showed the lowest value of MIC against *S. epidermidis* (0.24 mg/mL) and the highest value against *S. aureus* (0.89 mg/mL). The *n*-butanol fraction of stems showed the lowest value of MIC against *E. coli* (0.73 mg/mL) and the highest value against *S. epidermidis* (1.83 mg/mL). The *n*-hexane fraction of stems showed the highest value of MIC against *E. coli* (5.52 mg /mL) while the chloroform fraction of leaves showed the lowest value of MIC against *E. coli* (0.45 mg/mL). The ethyl acetate fraction of stems showed the maximum value of MIC against *S. epidermidis* (2.19 mg/mL).

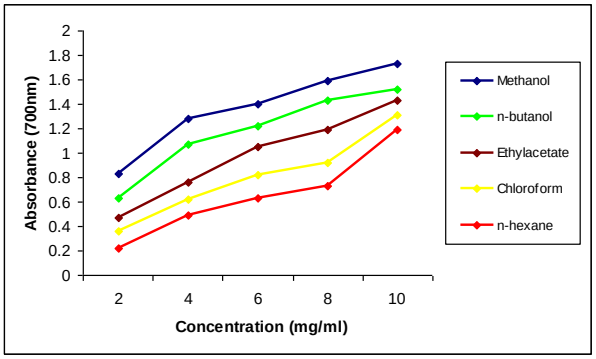

Fig. 1. Comparison of the reducing power activity of methanol extract and different fractions of leaves

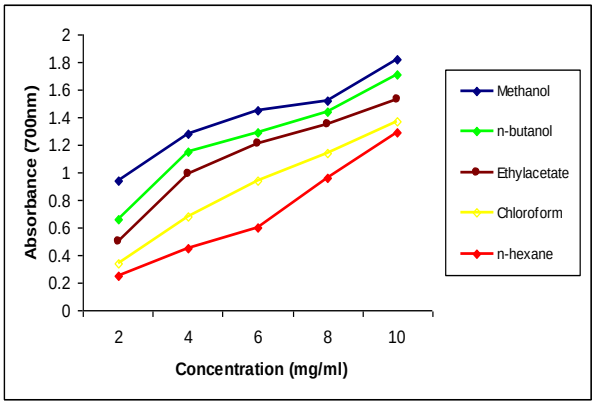

Fig. 2. Comparison of the reducing power activity of methanol extract and different fractions of roots

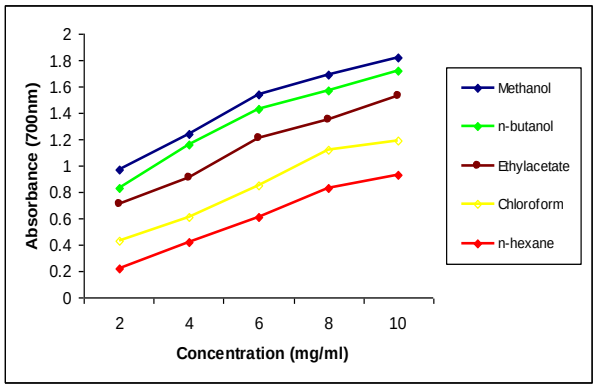

Fig. 3. Comparison of the reducing power activity of methanol extract and different fractions of stems

GC-MS Analysis

The GC-MS analysis of the *n*-hexane fractions of leaves, stems and roots from methanol extracts confirmed the presence of chemical components. The GC-MS chromatograms are shown in Figures (4-6) and results are presented in Tables (6-8). The volatile and non volatile fractions consisted of a mixture of different classes of compounds. In the *n*-hexane fraction of leaves 8 components representing 60.47% of the total fraction content were identified. The major constituents in the *n*-hexane fraction of leaves were found to be urs-12-en-24-oic acid 3-oxo-methyl ester (21.09%), urs-12-en-3β-ol-ethanoate, (17.58%), heneicosane (9.61%). The *n*-hexane fraction of stems revealed the presence of urs-12-en-24-oic acid 3-oxo-methyl ester (22.03%), 12-oleanen-3α-yl (8.74%) and β-amyrin (1.19%) as significant components. Hexadecanoic acid (3.02%), zeirone (5.46%), 12-oleanen-3-yl-ethanoate (15.6%) were found to be the dominant components in the *n*-hexane fraction of roots.

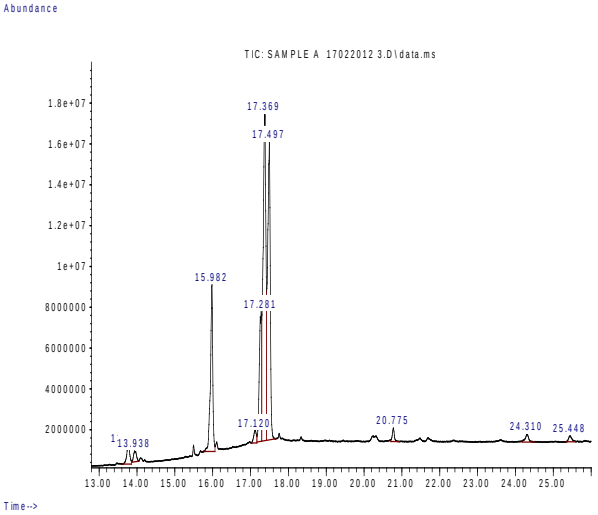

Fig 4. GC-MS chromatogram of the *n*-hexane fraction of plant leaves

Table 6: GC-MS analysis of the *n*-hexane fraction of plant leaves

Retention Time (min.)	Name of Compound	% Area
13.762	β-Amyrin; Olean-12-en-3 β-ol	1.281
13.938	α-Amyrin; Urs-12-en-3β-ol	0.898
15.982	12-Oleanen-3 α-yl-ethanoate	8.772
17.281	Vasicionolone	0.784
17.369	Urs-12-en-24-oic acid, 3-oxo-methyl ester (+)	21.095
17.497	Urs-12-en-3 β-ol-ethanoate	17.584
20.775	Heneicosane	9.615
24.310	Not identified	6.000
25.448	Stigmasterol	0.446

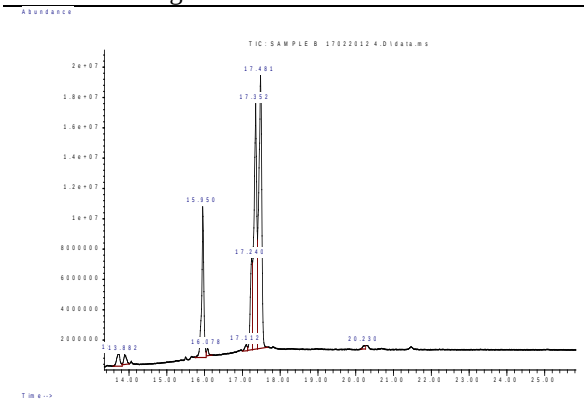

Fig. 5 GC-MS chromatogram of the *n*-hexane fraction of plant stems

Table 7: GC-MS analysis of the *n*-hexane fraction of plant stems

Retention Time (min.)	Name of Compound	% Area
13.882	Urs-12-en-3β–ol	0.821
15.95	12-Oleanen-3 α –yl	8.748
16.078	Olean-12-en-3 β-ol-ethanoate	0.349
17.112	Urs-12-en-3 β-ol-ethanoate	0.405
17.352	12-Oleanen-3 α-ethanoate	5.24
17.481	Urs-12-en-24-oic acid 3-oxo-methyl ester (+)	22.031
20.230	β-Amyrin	1.198

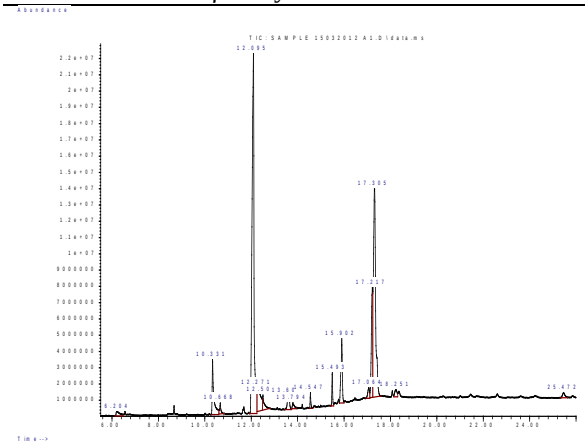

Fig. 6. GC-MS chromatogram of the *n*-hexane fraction of plant roots

Table 8. GC-MS analysis of the *n*-hexane fraction of plant roots

Retention Time (min.)	Name of Compound	% Area
6.204	Dodecanoic acid	0.589
10.331	Hexadecanoic acid	3.02
10.668	Eicosane	0.501
12.095	9,12-Octadecadienoic acid	0.331
13.61	Viminalol	1.016
13.794	α-Amyrin	0.378
14.547	Pyrrolidin-2-one 5-[2-butyrylethyl]	0.403
15.493	Di-(2-ethylhexyl)phthalate)	0.839
17.06	Urs-12-en-24-oic acid 3-oxo- methyl ester (+)	0.628
17.217	Zierone	5.465
17.305	12-Oleanen-3 α -yl-ethanoate	15.616
18.251	Friedooleanan-3-one	0.552
25.472	2-Amino-4-(3,4-dimethylphenyl) thiophene-3-carboxylic acidpropy ester	0.165

Acknowledgements: *The authors are highly thankful to Dr. Muhammad Athar Abbasi, Assistant Professor, Department of Chemistry, GC University Lahore, Pakistan, for providing the facility for GC-MS analysis.*

REFERENCES

1. F. Aslam, N. Rasool, M. Riaz, M. Zubair, K. Rizwan, M. Abbas, T. H. Bukhari, I. H. Bukhari, *Int. J. Phytomed.* **3**, 567 (2011).
2. M. R. Ahsan, K. M. Islam, M. E. Haque, M. A. Mossaddik, *World Journal of Agricultural Sciences* **5**, 617 (2009).
3. J. Sparrow, H. Gil, Subtropical Plants: A Practical Gardening Guide. Timber Press, Inc, Portland, Oregon, 2002.
4. E. M. Abdallah, A. E. Khalid, *Int. J. Chem. Biochem. Sci.* **1**, 1 (2012).
5. S. Adeel, F. Rehman, T. Gulzar, I. A. Bhatti, S. Qaiser, A. Abid, *Asian J. Chem.* **25**, 2739 (2012).
6. K. Rehman, S. Ashraf, U. Rashid, M. Ibrahim, S. Hina, T. Iftikhar, S. Ramzan, *Pak. J. Bot.* **45**, 391 (2013).
7. M. Riaz, N. Rasool, S. Rasool, U. Rashid, I. H. Bukhari, M. Zubair, M. Noreen, M. Abbas, *Asian J. Chem.* **25**, 5479 (2013).
8. H. O. Edeoga, D. E. Okwu, B. O. Mbaebie, *J. Biotechnol.* **4**, 685 (2005).
9. T. Okuda, *Phytochem.* **66**, 2012 (2005).
10. A. Chaovanalikit, R. E. Wrolstad, *J. Food Sci.* **69**, 67 (2004).
11. M. Riaz, N. Rasool, I. Bukhari, M. Shahid, F. Zahoor, M. Gilani, M. Zubair, *Afr. J. Microbiol. Res.* **6**, 5700 (2012).
12. V. Dewanto, X. Wu, K. K. Adom, R. H. Liu, *J. Agri. Food Chem.* **50**, 3010 (2002).
13. B. Bozin, D. Micica, N. Simin, G. Anackov, *J. Agric. Food Chem.* **54**, 1822 (2006).
14. G. C. Yen, P. D. Duh, D. Y. Chuang, *Food Chem.* **70**, 437 (2000).
15. T. Osawa, M. Namiki, *Agric. Biol. Chem.* **45**, 735 (1981).
16. NCCLS, Performance standards for antimicrobial disk susceptibility test by National Committee for Clinical Laboratory Standards (NCCLS), 6th ed. Wayne PA, M2-A6., 1997.
17. A. U. Rahman, M. I. Choudhary, *Pure Appl. Chem.* **73**, 555 (2001).
18. S. D. Sarker, L. Nahar, Y. Kumarasamy, *Methods* **42**, 321 (2007).
19. E. Derwich, Z. Benziane, R. Taouil, *Chemical Bulletin of "Politehnica" University of Timisoara, Romania* **55**, 103 (2010).
20. M. Riaz, N. Rasool, I. H. Bukhari, M. Shahid, M. Zubair, K. Rizwan, U. Rashid, *Molecules* **17**, 14275 (2012).

GC-MS ПРОФИЛИРАНЕ, АНТИОКСИДАНТНО И АНТИМИКРОБНО ИЗСЛЕДВАНЕ НА РАЗЛИЧНИ ЧАСТИ НА РАСТЕНИЕТО *Carissa grandiflora*

М. Абас[1], Н. Расул[1,*], М. Риаз[2], М. Зубаир [1], М. Аббас[3], Нур-ул-Хак[1], Н. Хаят[1]

[1] *Департамент по химия, Правителствен университетски колеж, Файсалабад 38000, Пакистан*
[2]*Катедра по химия в Университет на Саргода, Район на Жените, Файсалабад , Пакистан*
[3] *Департамент по химия и биохимия, Агрономически университет, Файсалабад38040, Пакистан*

Постъпила на 5 ноември, 2013 г.; коригирана на 2 януари, 2014 г.

(Резюме)

В настоящата работа се оценява биологичната активност на метанолови екстракти от листа, корени и стебла на растението *Carissa grandiflora* и техни фракции в разтворители с различна полярност (*n*-хексан, хлороформ, етилацетат и *n*-бутанол). Екстрактите и техните фракции съдържат значителни нива на общи фенолни производни (TPC) в интервала от 31.17 до 349.43 GAE, mg/100 g сухи вещества (листа), 38.85 до 269.81 еквивалент на галова киселина (GAE), mg/100 g сухи вещества (корени) и 40.18 до 241.11 GAE, mg/100 g сухи вещества (стебла). Общото съдържание на флавоноиди е от 59.14 до 284.99, 32.27 до 199.74 и 21.37 до 158.56 CE, mg/100 g сухи вещества съответно за листа, корени и стебла. Стойностите на IC_{50} в случай на DPPH радикал-премахваща активност за листа, корени и стебла са от 20.89 до 578.9, 12.28 до 325.31 и 6.15 до 941.4 mg/mL съответно. Процентното инхибиране на пероксидация по линоленова киселина е съответно от 11.34 до 46.7, 15.56 до 41.31 и 18.14 до 50.46 за листа, корени и стебла. Метаноловите екстракти от всички части на растенията показват най-висока редукционна активност в сравнение с другите фракции. Максимална антибактериална активност спрямо *S. aureus* показват екстрактите от стебла с етилацетат;. фракцията с *n*-butanol спрямо *E. coli* и меаноловите екстракти спрямо *S. epidermidis*. Щамът *C. albicans* показва най-голяма резистентност спрямо екстракта в етил ацетат от корени. GCMS-анализът на хексановата фракция от корени показва, ч в тази част на растението е обогатена с максимален брой биологично активни съединения.